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Determinants of urinary concentrations of dialkyl phosphates among pregnant women in Canada — Results from the MIREC study

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ABSTRACT

Organophosphate (OP) insecticides are commonly used in agriculture. Their use decreased in recent years as they were gradually replaced by other pesticides, but some OPs are still among the insecticides most used in Canada. Exposure to elevated levels of OPs during pregnancy has been associated with adverse birth outcomes and poorer neurodevelopment in children. The objective of the present study was to examine the relationship between the concentrations of OP pesticides urinary dialkyl phosphate (DAP) metabolites and various factors that are potential sources of exposure or determinants of DAP levels. In the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, six DAPs were measured in 1st trimester urine samples of 1884 pregnant women living in Canada. They were grouped into sums of dimethyl alkyl phosphates (DMAP) and diethyl alkyl phosphates (DEAP) for statistical analysis. We found that 93% of women had at least one DAP detected in their urine. Geometric means (GM) of specific gravity-corrected levels for urine dilution were 59 (95% CI 56–62) and 21 (95% CI 20–22) nmol/L for DMAP and DEAP, respectively. The following characteristics were significantly associated with higher urinary concentrations of DMAP or DEAP: higher education, nulliparous, normal pre-pregnancy body mass index, non-smoker, not fasting at sampling, winter season at sampling, and early and late day collection times. Dietary items that were significantly related with higher urinary concentrations included higher intake of citrus fruits, apple juice, sweet peppers, tomatoes, beans and dry peas, soy and rice beverages, whole grain bread, white wine and green and herbal teas. This study indicates that exposure to these compounds is quasi-ubiquitous. The factors associated with greater DAP levels identified here could be useful to regulatory agencies for risk analysis and management. However, some exposure misclassification might occur due to the single DAP measurement available, and to the presence of preformed DAPs in the environment.

1. Introduction

Organophosphate (OP) pesticides are primarily used as insecticides, mostly in agriculture but also to fight pests in residential and institutional settings (EPA, 2013). They are less persistent than the organochlorine pesticides that they replaced in the 1970s, but their acute toxicity in mammals is greater (Nauen et al., 2012). In turn, OPs are now being replaced by other pesticides, such as pyrethroids (EPA, 2011; van Balen et al., 2012); however, OPs are still commonly used today (Casida and Durkin, 2013).

For non-occupationally exposed individuals, the main route of exposure is ingestion of food, particularly contaminated fruits and vegetables (Oates et al., 2014). People living near farmlands can also be exposed by pesticides present in drinking water, air, and soil (Aggarwal et al., 2013; ATSDR, 2008). For occupationally-exposed individuals, the dermal route appears to be a significant route of exposure (An et al., 2014; Kongtip et al., 2013). OP pesticides are readily absorbed by the oral route, but less so dermally (Garfitt et al., 2002; Nolan et al., 1984). They do not accumulate appreciably in the body, and are rapidly metabolized and excreted in urine (Barr and Angerer, 2006). Most OP pesticides undergo a similar metabolism, starting with bioactivation creating the toxic oxon

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form, followed by detoxification producing dialkyl phosphate (DAP) metabolites (Barr and Angerer, 2006).

DAPs are non-specific urinary biomarkers of OP pesticides (i.e., each DAP can be generated from more than one OP pesticide); therefore, their measurement provides information about cumulative exposure to a large number of OP pesticides (Wessels et al., 2003). Furthermore, dimethyl OP pesticides produce only dimethyl alkyl phosphate (DMAP) metabolites, while diethyl OP pesticides produce only diethyl alkyl phosphate (DEAP) metabolites. Therefore, most studies group metabolites into these two classes. The urinary concentration of DAPs is indicative of exposure to OPs within hours or days of specimen sampling (Barr and Angerer, 2006). Results from the Canadian Health Measures Survey (CHMS) (2009–2011) indicate that 86% of the population had detectable levels of at least one DAP in urine (Health Canada, 2013). Females and children had higher levels than males and adults (Health Canada, 2013). It is important to note that preformed DAPs (residues of OP metabolites resulting from environmental degradation) can be found on food products after OP pesticide degradation and, thus, urinary DAPs are indicative of exposures to both OP pesticides and these preformed DAPs (CDC, 2009; EPA, 2013). Nonetheless, it is acknowledged that the measurement of urinary DAPs is useful to identify and compare levels of OP pesticide exposure in various populations, and with varying exposure conditions (Krieger et al., 2012; Sudakin and Stone, 2011).

While DAPs are metabolic products resulting in the detoxification of OP's, the oxon metabolites of OP's are bioactivation products, which are more active and thus more toxic than the parent compound. The oxon-metabolites of OP pesticides disrupt the nervous system by inhibiting acetylcholinesterase, the enzyme responsible for degrading acetylcholine in synapses, causing acetylcholine accumulation and resulting in excessive nerve stimulation (EPA, 2013). The mechanism of toxicity is less well understood for chronic low dose exposure. It could involve the parent compound instead of oxon-metabolite toxicity (Jameson et al., 2007) and mechanisms unrelated to acetylcholinesterase inhibition. Especially relevant for exposure to the developing fetus, multiple mechanisms have been reported to interfere with cellular processes that could directly affect brain morphogenesis, such as oxidative stress, growth factors, neurotransmitter and messenger systems (Lauder and Schambra, 1999; Slotkin, 2004; Slotkin and Seidler, 2007).

Pregnant women may have exposures to environmental chemicals which differ from those of the general population, since they experience notable physiological and behavioral changes during pregnancy (Abduljalil et al., 2012; Moya et al., 2014). Fetuses can be exposed to OP pesticides, as these pesticides can cross the placental barrier and have been measured in amniotic fluid samples (Bradman et al., 2003; Koutroulakis et al., 2014). Recent data indicate that the developing fetus might be particularly susceptible to OP toxicity. Exposure to OPs during pregnancy has been associated with shorter gestation duration, lower birth weight (Eskenazi et al., 2004; Rauch et al., 2012; Wang et al., 2012) and neurobehavioral impairment in young children (Bouchard et al., 2011; Gonzalez-Alzaga et al., 2013).

Few biomonitoring studies on OP pesticides have been reported using large cohorts of pregnant women, in particular during the first trimester when the fetus undergoes organogenesis, a critical developmental period. The objectives of the present study were to report the levels measured in Canadian pregnant women and to examine the relationship between urinary DAP levels in early pregnancy and various factors that may be potential sources of exposure or determinants of DAP levels.

2. Materials and methods

2.1. Study population

The Maternal-Infant Research on Environmental Chemicals (MIREC) cohort study includes 2001 women recruited in early pregnancy (<14 weeks gestation) from obstetric and prenatal clinics in 10 cities in 6 Canadian provinces. Pregnant women were approached between 2008 and 2011 and the participation rate was 39% from the 5108 eligible women (Arbuckle et al., 2013). Eligibility criteria included the ability to consent and to communicate in English or French, at least 18 years old, and planning to deliver at a participating study hospital. Exclusion criteria included having an adverse medical history: for either the mother (including major chronic disease, threatened abortion and illicit drug use) or the fetus (known major fetal abnormalities including chromosomal anomalies). The MIREC Study was designed to provide biomonitoring data on chemical exposure in pregnant women living in Canada. Details on the cohort have been previously reported (Arbuckle et al., 2013). The research protocol was reviewed and approved by the Health Canada Research Ethics Board and each of the ethics committees at the participating hospitals and research centers. Each woman enrolled in the study signed an informed consent form.

Of the 2001 women enrolled, 18 subsequently withdrew from the study. For the present study, we excluded women for whom urinary DAP or specific gravity measurements were not available ($n = 50$) and those with a multiple pregnancy ($n = 49$), leaving 1884 participants.

2.2. Urine collection and analysis

One spot urine sample was collected from each participant during the 1st trimester in 125 mL Nalgene® containers made of high-density polyethylene with polypropylene screw closure (Thermo-Fisher Scientific Inc., Rochester NY, USA), aliquoted into 30 mL Nalgene® containers, frozen at −20 °C within 2 h of collection, and shipped on dry ice to the coordinating center in Montreal where they were stored at −30 °C. Samples were then shipped in batches to the Centre de Toxicologie du Québec (CTQ) of the Institut National de Santé Publique du Québec (INSPQ) for determination of concentrations of DAP. Six DAP metabolites were assessed in each urine sample: three DMAP molecules (dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP) and dimethyl dithiophosphate (DMDTP)) and three DEAP molecules (diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP)).

DAPs were analyzed by GC–MS/MS and the analytical procedure was previously published (Health Canada, 2010). First, extraction involved hydrolysis with β-glucuronidase enzyme, derivation with pentafluorobenzyl bromide, and extraction of derivatized products using a mixture of dichloromethane:hexane (8:92). Then, analysis was done on an Agilent 6890 Network gas chromatograph (GC) (Agilent Technologies; Mississauga, Ontario, Canada) coupled to a Waters Quattro Micro GC mass spectrometer in tandem (MS/MS) (Waters; Milford, MA). The mass spectrometer was operated in Multiple Reaction Monitoring, using negative ion chemical ionization. The limits of detection (LOD) determination involved estimation of DAP concentrations yielding a signal-to-noise ratio (S/N) of 3, analysis of 10 replicates of a synthetic urine sample with DAP concentrations ranging from 4 to 10 times the previously estimated concentrations, and calculation of LODs using the standard deviations multiplied by three. The intra-day precision (repeatability) and the inter-day precision (reproducibility) of the method were from 3.7 to 8.2% and 3.5 to 9.9%, respectively, depending on the DAP. Quality control was assured by two internal reference materials (a noncertified ClinChek urine from Germany, and a homemade reference prepared from a pool of urine from exposed individuals), and the overall quality and accuracy of the method was monitored by the inter-laboratory program of the German External Quality Assessment Scheme (Erlangen, Germany). To assess urine dilution, specific gravity (SG) was measured in defrosted samples by a refractometer (UG-1, Atago / 3461, Atago U.S.A. Inc., Bellevue, WA).

2.3. Maternal sociodemographic and anthropometric characteristics and urine sampling conditions

Information from questionnaires and medical charts, as well as biological specimens, was collected during each trimester and at delivery. In the present study, we used data obtained from questionnaires administered by trained research nurses and assistants during the 1st trimester study visit. This questionnaire collected information on the characteristics of the participants (i.e., maternal age and education, household income, birth place, parity, pre-pregnancy body mass index (BMI) and smoking status) and on the conditions of urine collection (i.e., time of collection, time since last void, fasting status of sampling, and season of collection).

2.4. Maternal pesticide use, housing and diet

Part of the 1st trimester questionnaire included questions relating to maternal pesticide use and housing. Questions were asked about whether the women had used or had been exposed to pesticides during the current pregnancy, at work or at home for: 1) lawn and garden insects, 2) house plant insects, 3) pet fleas, and 4) residential pests (cockroaches, rodents, ants, etc.). Housing factors included questions about pet ownership, type of building of current residence, and having any rooms in the home with wall-to-wall carpets. In our sample, <1% of the data were missing for any given question.

Extensive data were collected on dietary habits during pregnancy. During the 1st trimester, information was collected on both alcoholic and non-alcoholic beverage consumption since the beginning of pregnancy (number of glasses or cups consumed per day, week or month). During the second trimester visit between 16 and 21 weeks of pregnancy, a one-month semi-quantitative food frequency questionnaire (FFQ) was administered including 46 food items listed in 6 subgroups (vegetables/fruits/meat, poultry, fish and alternatives/milk products/grain products/other foods). Only questions on grain products, fruits and vegetables were retained for the current analysis and included frequency of consumption (per day, week or month) as well as the serving size. As practiced by Health Canada, when small or large portions were reported, we applied a reduction or an inflation factor of 33% of the normal size, respectively. For each food and drink item, the consumption was categorized into three groups as evenly distributed as possible; however, for some drink items, only two categories were created as few women reported consuming any.

2.5. Statistical analysis

The analyses of factors associated with DAP levels in maternal urine were conducted using urinary concentrations which were corrected for specific gravity (SG) to account for urine dilution. Concentrations were corrected for SG using the following adapted formula (Just et al., 2010): $P_c = P_i[(SG_m - 1/SG_i - 1)]$, where P_c is the SG-corrected metabolite concentration, P_i is the observed metabolite concentration, SG_i is the SG of the ith urine sample and SG_m is the median SG for the cohort.

To calculate ΣDMAP and ΣDEAP levels, we converted mass concentrations in urine (μg/L) into molar concentrations (nmol/L) using the following molecular masses in g/mol: 126.05 (DMP), 142.11 (DMTP), 158.18 (DMDTP), 154.10 (DEP), 170.17 (DETP), and 186.24 (DEDTP). The robust regression on order statistics (ROS) semiparametric method was used to impute data below the LOD (Helsel, 2012) with R software. ROS combines parametric and nonparametric methods and is recommended for approximatively normal or lognormal distributions. Concentrations under the LOD were imputed for each of the 6 DAPs. Molar concentrations were log 10 transformed for the statistical analyses because the distributions were lognormal.

Univariate models were performed to identify the relationship between log 10 ΣDMAP and ΣDEAP levels and each participant's characteristics using analysis of variance (ANOVA). Beforehand, Lilliefors test was used to verify the normality of residual distributions for each factor category and Levene's test was used to test homogeneity of variances between categories of each factor. A p-value < 0.05 was used for statistical significance. Statistical analysis was performed using SPSS (version 22) and R (R Development Core Team).

3. Results

3.1. Cohort profile and urinary DAP levels

The sociodemographic characteristics of the 1884 participants included in the present study are outlined in Table 1. Most women in this cohort were highly educated (62% had a university degree), were non-smokers (61% never smoked), and 73% were 30 years of age and older (mean 32.6 ± 5.1, range 18–48 years). The median gestational age at the time of women's recruitment into the study, which is when urine was collected for DAP measurement, was 12.0 weeks (range

Table 1
Geometric means for urinary concentrations of DAP (nmol/L) corrected for specific gravity with respect to maternal and urine sampling characteristics in the MIREC Study (2008–2011).

Maternal and urine sampling factors	n (%)	GM (nmol/L, 95% CI)	
		ΣDMAP	ΣDEAP
Maternal characteristics			
Maternal age (years)		p = 0.19	p = 0.39
< 25	101 (5)	52 (41–66)	18 (41–66)
25–29	405 (21)	59 (53–66)	21 (53–66)
30–34	667 (35)	62 (58–68)	21 (58–68)
≥35	711 (38)	56 (52–60)	21 (52–60)
Education		p = **0.02**	p = **0.02**
High school	168 (9)	49 (41–59)	19 (16–21)
College diploma	545 (29)	56 (51–61)	20 (18–21)
University degree	1169 (62)	62 (58–66)	22 (21 − 23)
Household income ($)		p = 0.37	p = **0.04**
≤50,000	328 (18)	56 (49–63)	19 (17–21)
50,001–100,000	752 (42)	62 (57–66)	22 (20 − 23)
>100,000	716 (40)	58 (54–63)	21 (20 − 22)
Birth place		p = 0.53	p = 0.10
Canada	1528 (81)	58 (55–61)	21 (20 − 21)
Other	356 (19)	61 (54–68)	22 (20–25)
Parity		p < **0.001**	p < **0.01**
0	853 (44)	68 (63–73)	22 (21–24)
1	780 (40)	53 (49–57)	20 (19–21)
>1	298 (15)	50 (44–56)	19 (17–21)
Pre-pregnancy BMI (kg/m^2)		p = **0.01**	p < **0.01**
<25	1123 (63)	62 (59–66)	22 (21–23)
25–29	382 (22)	53 (47–59)	20 (18–22)
≥30	265 (15)	54 (48–62)	19 (17–21)
Smoking status		p = **0.02**	p < **0.01**
Never	1144 (61)	61 (58–65)	21 (20–22)
Current[a]	226 (12)	49 (42–57)	17 (15–20)
Former	512 (27)	58 (52–63)	22 (20–23)
Sampling characteristics			
Time of urine collection		p = **0.001**	p = 0.97
06:00–09:00	27 (1)	65 (42–101)	23 (15–33)
09:00–12:00	814 (43)	56 (52–60)	21 (19–22)
12:00–15:00	637 (34)	55 (51–60)	22 (19–22)
15:00–18:00	366 (19)	69 (62–78)	21 (19–23)
18:00–24:00	38 (2)	87 (64–119)	20 (16–26)
Time since last urination (min)		p = 0.95	p = 0.17
< 75	440 (23)	60 (54–66)	20 (18–22)
75–119	359 (19)	58 (52–64)	22 (20–24)
120–169	539 (29)	58 (53–64)	20 (18–22)
≥ 170	453 (24)	59 (53–66)	22 (20–23)
Fasting status		p < **0.001**	p = 0.18
No	1822 (98)	59 (56–62)	21 (20–22)
Yes	39 (2)	33 (23–46)	17 (13 − 23)
Season of collection		p = **0.05**	p = **0.001**
Fall (Sept. 21–Dec. 20)	550 (29)	53 (48–58)	19 (18–20)
Winter (Dec. 21–Mar. 19)	450 (24)	63 (57–70)	25 (23–27)
Spring (Mar. 20–Jun. 20)	443 (24)	62 (56–68)	22 (20–24)
Summer (Jun. 21–Sept. 20)	441 (23)	59 (53–65)	19 (17–20)

[a] Includes women who quit smoking during current pregnancy.

Table 2
Descriptive statistics on urinary concentrations of DAP uncorrected and corrected for specific gravity in women from the MIREC Study (n = 1884; 2008–2011).

Metabolites	LOD		%	Uncorrected DAP concentrations (nmol/L)				SG-corrected DAP concentrations (nmol/L)			
	nmol/L	μg/L	<LOD	GM (95% CI)	Median (95% CI)	95th perc.	Max.	GM (95% CI)	Median (95% CI)	95th perc.	Max.
DMP	7.93	1.00	21	23 (22–24)	23 (21–25)	167	1507	26 (25–27)	26 (25–28)	134	852
DMTP	4.22	0.60	20	20 (19–22)	21 (19–24)	239	690	23 (21–24)	24 (22–26)	210	903
DMDTP	1.90	0.30	49	2 (2–2)	2 (2–2)	38	518	2 (2–2)	2 (2–3)	33	250
DEP	6.49	1.00	23	14 (14–15)	14 (14–15)	84	22,064	16 (15–17)	16 (15–17)	66	13,658
DETP	3.53	0.60	47	3 (3–3)	3 (2–3)	25	294	3 (3–3)	4 (3–4)	21	224
DEDTP	1.61	0.30	98	NC[a]	NC[a]	NC[a]	35	NC[a]	NC[a]	NC[a]	22
ΣDMAP[b]	1.90	0.30	13	52 (49–55)	52 (49–56)	440	1611	59 (56–62)	60 (56–64)	346	1310
ΣDEAP[c]	1.61	0.30	20	19 (18–19)	18 (17–19)	113	22,076	21 (20–22)	21 (20–22)	86	13,666
ΣDAP[d]	1.61	0.30	7	78 (74–82)	78 (74–83)	538	22,516	88 (84–92)	86 (82–91)	407	13,939

[a] NC: not calculated because >50% of values were below the LOD.
[b] ΣDMAP = DMP + DMTP + DMDTP.
[c] ΣDEAP = DEP + DETP + DEDTP.
[d] ΣDAP = ΣDMAP + ΣDEAP.

2.7–15.9 weeks). Further details on the MIREC study population are reported elsewhere (Arbuckle et al., 2013).

Descriptive statistics for DAP levels are shown in Table 2, and results expressed as μg/L are shown in the Supplemental Material. Among women participating, 93% had at least one DAP metabolite detected in urine among the six DAPs analyzed. The three most prevalent DAPs in maternal urine were DMP, DMTP and DEP, with frequencies of detection of 79%, 80% and 77%, respectively. DMDTP, DETP and DEDTP had detection frequencies of 51%, 53% and 2%, respectively. For the DMAP metabolites, the geometric mean (GM) SG-corrected DMP concentration (26 nmol/L) was similar to that of DMTP (23 nmol/L), while the GM SG-corrected DMDTP concentration was much lower (2 nmol/L). The GM for the sum of these three metabolites (ΣDMAP) was 59 nmol/L. For the DEAP metabolites, the GM SG-corrected concentration of DEP (16 nmol/L) was higher than that of DETP (3 nmol/L); it was not calculated for DEDTP because of the low detection frequency (2%). The geometric mean of the sum of these three metabolites (ΣDEAP) was 21 nmol/L.

3.2. Relation between urinary DAP concentrations, maternal characteristics and sample collection conditions

When maternal characteristics (including age, education, income, birth place, parity, pre-pregnancy BMI and smoking status) were examined, higher urinary concentrations of ΣDMAP and ΣDEAP (Table 1) were associated with several factors, including education level, parity, pre-pregnancy BMI and smoking status. DAP concentrations increased with education level for both ΣDMAP ($GM_{HighSchool}$ = 49 and $GM_{University}$ = 62 nmol/L) and ΣDEAP ($GM_{HighSchool}$ = 19 and $GM_{University}$ = 22 nmol/L). DAP concentrations decreased with parity for both ΣDMAP (GM_0 = 68 and $GM_{>1}$ = 50 nmol/L) and ΣDEAP (GM_0 = 22 and $GM_{>1}$ = 19 nmol/L), and decreased with pre-pregnancy BMI for both ΣDMAP ($GM_{<25}$ = 62 and $GM_{\geq 30}$ = 54 nmol/L) and ΣDEAP ($GM_{<25}$ = 22 and $GM_{\geq 30}$ = 19 nmol/L). In addition, women who never smoked had significantly higher DAP concentrations than current smokers for both ΣDMAP (GM_{Never} = 61 and $GM_{Current}$ = 49 nmol/L) and ΣDEAP (GM_{Never} = 21 and $GM_{Current}$ = 17 nmol/L); former smokers had similar ΣDMAP and ΣDEAP concentrations to women who never smoked. Finally, ΣDEAP concentrations were significantly lower in the lower income group ($GM_{\leq 50.000}$ = 19 and $GM_{50–100.000}$ = 22 nmol/L). No significant differences were observed in ΣDMAP and ΣDEAP concentrations with respect to maternal age and maternal birth place.

With respect to urine sampling characteristics (Table 1), DAP concentrations were significantly different according to the season at urine collection, with levels higher in the winter than the fall for both ΣDMAP (GM_{Winter} = 63 and GM_{Fall} = 53 nmol/L) and ΣDEAP (GM_{Winter} = 25 and GM_{Fall} = 19 nmol/L). ΣDMAP concentrations differed significantly by time of the day at urine collection, with levels lower in the middle of the day than earlier or later in the day (e.g., $GM_{12:00–15.00}$ = 55 and $GM_{15:00–18.00}$ = 69 nmol/L). ΣDMAP concentrations were significantly lower in samples collected from fasting women (GM_{Yes} = 33 and GM_{No} = 59 nmol/L), but there were very few fasting participants (2%). No significant difference was observed in ΣDMAP and ΣDEAP concentrations with respect to time since last urination.

3.3. Relation between urinary DAP concentrations and characteristics related to pesticide use and dwelling characteristics

Few women reported using or having been exposed to pesticides since they were pregnant (2–8%, depending on the type of pesticide use) (Table 3). Although not statistically significant, there was a trend for higher ΣDMAP concentrations in women who reported pesticide use for residential pests (e.g., cockroaches, rodents, ants, etc.) (GM_{Yes} = 69 and GM_{No} = 58 nmol/L). No difference was observed in ΣDMAP concentrations with respect to type of housing, pet ownership, or wall-to-wall carpet in the house. Finally, ΣDEAP concentrations were not associated with pesticide use or dwelling characteristics.

Table 3
Geometric means for urinary concentrations of DAP (nmol/L) corrected for specific gravity with respect to pesticide use and dwelling characteristics since the beginning of current pregnancy for women from the MIREC Study (2008–2011).

Pesticide use and dwelling characteristics	n (%)	GM (nmol/L, 95% CI)	
		ΣDMAP	ΣDEAP
Pesticide use for:			
Lawn and garden insects		p = 0.51	p = 0.56
No	1812 (97)	59 (56–62)	21 (20–22)
Yes	60 (3)	64 (50–83)	20 (16–24)
House plant insects		p = 0.28	p = 0.74
No	1844 (98)	59 (56–62)	21 (20–22)
Yes	34 (2)	48 (33–70)	22 (17–28)
Pet fleas		p = 0.27	p = 0.76
No	1750 (93)	59 (56–62)	21 (20–22)
Yes	125 (7)	53 (44–64)	20 (17–24)
Residential pests[a]		p = 0.06	p = 0.67
No	1723 (92)	58 (55–61)	21 (20–22)
Yes	155 (8)	69 (58–82)	21 (19–25)
Owning a dog or a cat		p = 0.10	p = 0.66
No	906 (48)	61 (57–66)	21 (20–22)
Yes	975 (52)	56 (53–60)	21 (20–22)
Type of residence		p = 0.90	p = 0.88
Semi or fully-detached house	1513 (80)	59 (56–62)	21 (20–22)
Apartment building	367 (20)	58 (52–66)	21 (19–23)
Wall-to-wall carpets in any room		p = 0.40	p = 0.62
No	853 (45)	60 (56–65)	21 (20–22)
Yes	1027 (55)	58 (54–61)	21 (20–22)

[a] Cockroaches, rodents, ants, etc.

3.4. Relation between urinary DAP concentrations and food, drinks, and alcohol

The consumption of several vegetables and fruits was associated with significant differences in urinary DAP concentrations (Table 4). DAP concentrations increased significantly with citrus fruit consumption for both ΣDMAP (GM_{Low} = 53 and GM_{High} = 71 nmol/L) and ΣDEAP (GM_{Low} = 19 and GM_{High} = 23 nmol/L). The concentrations also increased significantly with apple juice consumption for both ΣDMAP (GM_{Low} = 52 and GM_{High} = 70 nmol/L) and ΣDEAP (GM_{Low} = 19 and GM_{High} = 24 nmol/L). ΣDMAP concentrations increased significantly with higher consumption of sweet peppers (GM_{Low} = 50 and GM_{High} = 69 nmol/L), tomatoes (GM_{Low} = 54 and GM_{High} = 67 nmol/L), beans and dry peas (GM_{Low} = 54 and GM_{High} = 65 nmol/L). Although not statistically significant, there was a trend for higher ΣDMAP concentrations with broccoli consumption (GM_{Low} = 55 and GM_{High} = 62 nmol/L), and for higher ΣDEAP concentrations with raw spinach consumption (GM_{Low} = 20 and GM_{High} = 23 nmol/L). There was no difference in ΣDMAP and ΣDEAP concentrations with respect to tomato or vegetable juice, potatoes, or grape juice.

The results for grain products are shown in Table 5. ΣDMAP concentrations increased significantly with increased consumption of soy and rice beverage (GM_{Low} = 58 and GM_{High} = 70 nmol/L) and whole grain bread (GM_{Low} = 55 and GM_{High} = 65 nmol/L), whereas it decreased significantly with increased white bread consumption (including rolls, buns, bagels, pita and tortillas) (GM_{Low} = 65 and GM_{High} = 55 nmol/L). ΣDEAP concentrations were significantly lower with higher pasta consumption (GM_{Low} = 22 and GM_{High} = 19 nmol/L). There was a trend for higher levels with higher cold cereal consumption for both ΣDMAP (GM_{Low} = 55 and GM_{High} = 64 nmol/L) and ΣDEAP (GM_{Low} = 20 and GM_{High} = 22 nmol/L), and with consumption of snack bars made of cereals for ΣDMAP (GM_{Low} = 57 and GM_{High} = 65 nmol/L). ΣDMAP concentrations differed significantly with cake consumption (including muffins, pies, pastries and doughnuts), but there was no clear relation with the level of intake. No difference was observed in ΣDMAP and ΣDEAP concentrations with respect to various consumption patterns for hot cereal, crackers and cookies.

Urinary DAP concentrations increased significantly with increased white wine consumption (Table 6) for both ΣDMAP (GM_{Low} = 57 and GM_{High} = 66 nmol/L) and ΣDEAP (GM_{Low} = 20 and GM_{High} = 23 nmol/L). ΣDMAP concentrations increased significantly with increased green tea consumption (GM_{Low} = 57 and GM_{High} = 65 nmol/L), and there was a trend for higher ΣDMAP concentrations with increased consumption of red wine (GM_{Low} = 57 and GM_{High} = 64 nmol/L) and beer (GM_{Low} = 57 and GM_{High} = 65 nmol/L). ΣDEAP concentrations increased significantly with increased herbal tea consumption (GM_{Low} = 20 and GM_{High} = 22 nmol/L), whereas they

Table 4
Geometric means for urinary concentrations of DAP (nmol/L) corrected for specific gravity with respect to vegetable and fruit consumption, of women from the MIREC Study (2008–2011).

Vegetable and fruit consumption	n (%)	GM (nmol/L, 95% CI)	
		ΣDMAP	ΣDEAP
Broccoli[a]		p = 0.08	p = 0.34
Low	667 (38)	55 (51–60)	20 (19–22)
Medium	549 (31)	62 (55–68)	22 (20–24)
High	529 (30)	62 (57–68)	21 (20–23)
Raw spinach[a]		p = 0.12	p = 0.07
Low	925 (53)	57 (53–61)	20 (19–22)
Medium	316 (18)	65 (58–73)	20 (19–23)
High	504 (29)	61 (56–67)	23 (21–24)
Sweet peppers[a]		p < **0.001**	p = 0.37
Low	587 (34)	50 (46–55)	20 (19–22)
Medium	653 (37)	61 (56–66)	21 (20–22)
High	505 (29)	69 (64–76)	22 (20–24)
Tomatoes[a]		p = **0.003**	p = 0.15
Low	682 (39)	54 (49–59)	21 (19–22)
Medium	633 (36)	61 (56–66)	20 (19–22)
High	430 (25)	67 (61–74)	23 (21–24)
Tomato or vegetable juice[a]		p = 0.83	p = 0.66
Low	1179 (68)	59 (55–63)	21 (20–22)
Medium	283 (16)	59 (52–67)	22 (20–24)
High	283 (16)	62 (55–70)	21 (29–23)
Potatoes[a]		p = 0.92	p = 0.29
Low	633 (36)	59 (54–64)	22 (20–24)
Medium	555 (32)	60 (55–66)	20 (19–22)
High	557 (32)	60 (55–65)	21 (19–22)
Beans and dry peas[a]		p = **0.02**	p = 0.13
Low	658 (38)	54 (50–59)	20 (19–22)
Medium	531 (30)	61 (56–66)	21 (19–22)
High	533 (31)	65 (59–70)	22 (21–24)
Citrus fruits[a]		p < **0.001**	p < **0.001**
Low	688 (39)	53 (49–58)	19 (18–21)
Medium	524 (30)	58 (53–63)	21 (20–23)
High	533 (31)	71 (65–77)	23 (22–25)
Apple Juice[b]		p < **0.001**	p < **0.001**
Low	866 (46)	52 (48–55)	19 (18–20)
Medium	550 (29)	62 (56–68)	21 (19–22)
High	463 (25)	70 (63–77)	24 (23–26)
Grape juice or cocktail[b]		p = 0.53	p = 0.54
Low	1393 (74)	58 (55–61)	21 (20–22)
Medium	258 (14)	60 (53–69)	21 (19–24)
High	228 (12)	63 (55–71)	22 (20–25)

[a] From FFQ administered during 2nd trimester.
[b] From questionnaire administered during 1st trimester.

Table 5
Geometric means for urinary concentrations of DAP (nmol/L) corrected for specific gravity with respect to grain products consumption, of women from the MIREC Study (2008–2011).

Grain products consumption[a]	n (%)	GM (nmol/L, 95% CI)	
		ΣDMAP	ΣDEAP
Soy and rice beverages		p = **0.006**	p = 0.11
Low	1475 (85)	58 (55–61)	21 (20–22)
High	270 (15)	70 (62–79)	23 (21–25)
Cold cereal		p = **0.05**	p = 0.06
Low	581 (33)	55 (50–60)	20 (18–21)
Medium	531 (30)	59 (54–64)	22 (20–23)
High	633 (36)	64 (59–70)	22 (20–23)
Hot cereal		*p* = 0.71	*p* = 0.95
Low	1033 (59)	60 (56–64)	21 (20–22)
Medium	196 (11)	56 (48–65)	21 (19–24)
High	516 (30)	60 (55–66)	21 (19–23)
White bread		p = **0.03**	p = 0.15
Low	590 (34)	65 (59–70)	21 (20–23)
Medium	572 (33)	59 (54–64)	22 (20–24)
High	583 (33)	55 (50–60)	20 (19–21)
Whole grain bread		p = **0.02**	p = 0.15
Low	958 (55)	55 (51–59)	20 (19–22)
High	787 (45)	65 (61–70)	22 (21–23)
Pasta		p = 0.45	p = **0.03**
Low	641 (37)	60 (55–65)	22 (20–23)
Medium	567 (32)	62 (56–67)	22 (21–24)
High	537 (31)	57 (52–62)	19 (18–21)
Crackers		p = 0.95	p = 0.68
Low	605 (35)	59 (54–64)	21 (19–22)
Medium	660 (38)	60 (55–65)	21 (19–22)
High	480 (28)	59 (54–65)	22 (20–23)
Cookies		p = 0.23	p = 0.60
Low	705 (61)	62 (58–68)	21 (19–22)
Medium	459 (39)	58 (53–64)	21 (19–22)
High	581 (50)	57 (52–62)	22 (20–23)
Cake		p = **0.04**	p = 0.74
Low	575 (33)	57 (52–62)	21 (19–22)
Medium	583 (33)	65 (60–71)	21 (20–23)
High	587 (34)	57 (52–62)	21 (20–23)
Snack bars made of cereals		p = 0.10	p = 0.20
Low	916 (52)	57 (53–61)	22 (20–23)
Medium	353 (20)	59 (53–65)	20 (19–21)
High	476 (27)	65 (59–71)	21 (19–23)

[a] From FFQ administered during 2nd trimester.

Table 6
Geometric means for urinary concentrations of DAP (nmol/L) corrected for specific gravity with respect to drinks consumption of pregnant women from the MIREC Study (2008–2011).

Drinks consumption[a]	n (%)	GM (nmol/L, 95% CI)	
		ΣDMAP	ΣDEAP
Regular and decaffeinated coffee		p = 0.16	p = 0.36
Low	875 (47)	61 (56–65)	21 (20–23)
Medium	422 (22)	61 (55–67)	21 (19–23)
High	579 (31)	55 (50–59)	20 (19–21)
Regular tea		p = 0.25	p = 0.34
Low	1112 (59)	60 (56–64)	21 (20–22)
High	767 (41)	57 (52–61)	20 (19–22)
Green tea		p = **0.02**	p = 0.82
Low	1451 (77)	57 (54–60)	21 (20–22)
High	429 (23)	66 (59–72)	21 (19–23)
Herbal tea		p = 0.14	p = **0.04**
Low	1171 (62)	57 (54–61)	20 (19–21)
High	707 (38)	62 (57–67)	22 (21–23)
White wine		p = **0.03**	p = **0.04**
Low	1577 (84)	57 (54–60)	20 (20–21)
High	302 (16)	66 (59–75)	23 (21–25)
Red wine		p = **0.05**	p = 0.57
Low	1432 (76)	57 (54–60)	21 (20–22)
High	445 (24)	64 (58–71)	21 (20–23)
Beer		p = 0.07	p < **0.001**
Low	1563 (83)	57 (55–61)	21 (20–22)
High	315 (17)	65 (57–74)	19 (18–22)

[a] From questionnaire administered during 1st trimester.

decreased significantly with increased beer consumption (GM_{Low} = 21 and GM_{High} = 19 nmol/L). No difference in urinary DAP concentrations were observed with various coffee and regular tea consumption patterns.

4. Discussion

In this study, we report urinary DAP concentrations, which are metabolites of OP pesticides, measured early in pregnancy among women from the MIREC cohort. We found that 93% of women had at least one DAP detected in their urine, similar to the general population living in Canada at 91% (Ye et al., 2015). Looking individually at the three most often detected DAPs (DMP, DMTP, DEP), levels in this study were similar to nonpregnant women living in Canada for DMP (GM_{MIREC} = 23 and GM_{CHMS} = 21 nmol/L) and DEP (GM_{MIREC} = GM_{CHMS} = 14 nmol/L), but slightly higher for DMTP (GM_{MIREC} = 20 and GM_{CHMS} = 13 nmol/L) (Health Canada, 2013). In the present study, levels were lower than those found in pregnant women from an agricultural community in California (Bradman et al., 2005) for ΣDMAP (GM_{MIREC} = 52 and $GM_{CHAMACOS}$ = 83 nmol/L), and similar for ΣDEAP (GM_{MIREC} = 19 and $GM_{CHAMACOS}$ = 17 nmol/L). While levels in pregnant Canadian women were slightly higher than those previously reported in pregnant women from the general population living in USA for DMTP (GM_{MIREC} = 20 and GM_{NHANES} = 17 nmol/L) (Woodruff et al., 2011), levels were lower than those noted in pregnant women in China for DMP (GM = 143 nmol/L), DMTP (GM = 60 nmol/L) and DEP (GM = 46 nmol/L) (Zhang et al., 2014).

Eighteen OP pesticides were registered in Canada during the time of the present study (2009–2011) (Health Canada, 2013). This included eight dimethyl OPs (azinphos-methyl, dimethoate, malathion, and phosmet, dichlorvos, naled, tetrachlorvinphos and trichlorfon) producing DMAP metabolites, six diethyl OPs (chlorpyrifos, coumaphos, diazinon, phorate, phosalone and terbufos) producing DEAP metabolites, and four OPs not producing DAP metabolites (acephate, bensulide, methamidophos and propetamphos). Among insecticides, chlorpyrifos was the third most sold insecticide active ingredient in 2012 (between 100,000 and 500,000 kg of active ingredient) after mineral oil and hydrogen peroxide. Among OP pesticides most sold in 2012, chlorpyrifos was followed by dimethoate, malathion and diazinon (Health Canada, 2012). These pesticides are widely used in agricultural settings.

In Canada, a large fraction of certain fruits and vegetables have detectable pesticide residues (CFIA, Canadian Food Inspection Agency, 2008–2012 reports). For instance, >33% of samples for the following foods had multiple pesticides detected: orange, strawberry, celery, pea, bean, spinach, grape, apricot, apple, cucumber, lettuce leaf and potato. Some of these produce were associated with greater urinary DAP levels in the present study (e.g. citrus fruits, apple juice, sweet peppers, beans and peas). Based on monitoring data for pesticide residues in fresh fruit and vegetables available for consumption in Canada, chlorpyrifos, phosmet, methamidophos, malathion, profenofos and acephate were among the pesticides implicated in the pesticide residue violations observed between 2008 and 2012 (e.g., 0.26% and 0.14% of samples tested for chlorpyrifos and for phosmet had residues greater than the maximum residue limit). Pesticide residue violations are more common in imported produce than in produce from Canada (0.9–2% versus 0.2–0.8%, respectively). In the present study, higher DMAP concentrations were measured in samples collected during winter, a season when Canadians rely greatly on imported produce. In addition, lower DMAP concentrations were measured when women were fasting (8–12 h before sampling, water allowed), supporting the substantial amount of data already showing that diet is an important source of OP pesticides exposure.

In MIREC, DMAPs were more frequently detected than DEAPs, and they were present in higher concentrations. This is consistent with findings in other studies (Eskenazi et al., 2007; Health Canada, 2013; Ye et al., 2008; Zhang et al., 2014). This may indicate that levels of exposure are greater in the population for dimethyl OP pesticides such as malathion and dimethoate, the dimethyl OP pesticides most often used in Canada. Also, the longer environmental half-lives of DMAP might contribute to this finding (Ye et al., 2008).

In the present study, women had higher DAP urinary concentrations if they were more educated, had a higher income, did not smoke and had a pre-pregnancy BMI below 25 kg/m^2. These characteristics might very well be associated with a greater awareness on the importance of good nutrition, which includes higher consumption of fruits and vegetables. A higher income also allows individuals to make this dietary choice (Kamphuis et al., 2006; Raine, 2005).

Pesticide use was seldom reported by MIREC women. If reported, it was most frequently for pet fleas and residential pests; however, the reporting time period for this question was only within the few weeks after the beginning of their pregnancy. Although not statistically significant, there was a trend for higher ΣDMAP levels in women reporting pesticide use for residential pests. This was unexpected since no dimethyl OP pesticide is registered for indoor residential use in Canada, but they are for lawn and garden (e.g., malathion for flies and garden insects, including mosquitoes and spiders). Instead, it was expected ΣDEAP levels to be higher in women reporting pesticide use for residential pests, since diethyl OP pesticide are registered for indoor residential use in Canada (e.g., chlorpyrifos for ants). In contrast to the findings of a previous study (Valcke et al., 2006), we observed no difference in DAP levels between women living in a house with a pet or not.

Our results showed that several fruits and vegetables consumed were associated with higher DAP levels. For instance, ΣDMAP levels were higher in women reporting higher intake of sweet peppers (38% higher than the lowest intake group), tomatoes (24%), beans and dry peas (20%) and citrus fruits (34%). This observation is consistent with other population-based studies where higher exposures to OP pesticides were related to fruit and vegetable consumption (Berman et al., 2013; McKelvey et al., 2013; Ye et al., 2015). Higher ΣDMAP levels were also observed with soy and rice beverages (21%) and whole grain bread (18%) consumption. On the other hand, higher intakes of pasta and white bread were associated with lower DAP levels. A possible explanation is that individuals who eat white bread presumably eat less

whole grain bread and possibly fewer fruits and vegetables, the latter being associated with higher DAP exposure. Ye and collaborators (2015) reported no relationship between ΣDMAP levels and consumption of grain and grain-based products. In their analysis, grain and grain-based products were studied as a whole group, whereas in the present study we analyzed each of these products separately. An increase of DAP levels was also observed with increased white wine (16% with ΣDMAP and 15% with ΣDEAP), green tea (16% with ΣDMAP) and herbal tea (10% with ΣDEAP) consumption. Colapinto et al. (2015) found no association with tea consumption when looking at individual urinary DAP concentrations. However, pesticide residues are often found in tea, with 41% of the samples presenting residues concentrations above the maximum limit in a recent survey (CFIA, Canadian Food Inspection Agency, 2009–2010a, b).

A strength of the MIREC study is the large sample size, with participants drawn from a diverse pregnant population across Canada enabling the more accurate estimation of exposure ranges. This is one of the largest sample sizes reported for measurements of DAPs during pregnancy, internationally. Another strength is the adjustment for urine dilution with SG. This method is more appropriate to assess exposure during pregnancy than the common approach using creatinine. Indeed, it has been shown that creatinine urinary levels are affected by various factors such as age, muscular mass and pregnancy (Boeniger et al., 1993).

Several factors must be considered when interpreting our results. As DAPs are non-specific metabolites, the identities of the specific OP parent pesticide(s) remains unknown. Also, only one spot urine sample was collected from each participant during the 1st trimester (the laboratory costs preclude collection of multiple samples per woman in most large studies). Because most OP pesticides have short biological half-lives (WHO, World Health Organization, 1986), urinary DAP concentrations reflect recent exposure. Serial urine specimens in pregnant women in the Netherlands showed moderate to low reliability, with an intraclass correlation coefficient of 0.3 for total DAPs (values near one indicating high reliability) (Spaan et al., 2015). The collection of several urine samples per woman would have provided a more robust estimation of long-term exposure to these compounds. Furthermore, the population is also exposed to DAPs present in the environment (preformed DAPs), therefore urinary DAP levels might be interpreted as an indication of the general level of a population exposure to OP pesticides or to preformed DAPs. The relative contribution of preformed DAP to total maternal urinary levels of DAP could not be ascertained in this investigation. Some of the data on the diet were collected a few months after urine sampling, possibly impacting the results if dietary changes occurred. It is possible that some findings occurred by chance since we conducted multiple tests of statistical association. Finally, we should be cautious when generalizing the factors associated with increased cumulative exposure to OP's in pregnant women in Canada since the cohort of women in the present study was healthier, more educated, smoked less and was older on average than pregnant women in the general population in Canada (Arbuckle et al., 2013).

In conclusion, the present study identified the following characteristics that were significantly associated with higher urinary concentrations of DMAP or DEAP: higher education, nulliparous, normal pre-pregnancy body mass index, non-smoker, fasting, winter season at sampling and early and late day collection times. Some of the maternal characteristics associated with higher DAP urinary concentrations might be associated with a greater awareness of the importance of good nutrition, such consuming fresh produce, which is also associated with greater pesticide exposure. Dietary items significantly related with higher concentrations included higher intake of citrus fruits, apple juice, sweet peppers, tomatoes, beans and dry peas, soy and rice beverages, whole grain bread, white wine, green and herbal tea. The factors associated with greater DAP levels identified here could be useful to regulatory agencies for risk analysis and management.

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